# NEW YORK PATHOLOGICAL SOCIETY

## ABSTRACTS OF PAPERS AND DISCUSSION

Presented at the Meeting of May 23, 1957, at The New York Academy of Medicine

## *An Investigation of the Relation between Immediate and Delayed Hypersensitivity as Illustrated by Local Lesions in Rabbits*

PHILIP G. H. GELL

Department of Pathology, University of Birmingham, England

Study of the types of cells specifically involved in hypersensitivity reactions can give some insight into the mechanism of their causation and development, and throw light upon two problems: first, what is the "sensitized" cell in delayed sensitivity, and secondly, is there any direct continuity between the cell that takes up antigen, the cell that becomes "sensitized", and the cell that produces and secretes antibody?

It is first necessary to consider the "architecture" of the various sorts of hypersensitivity lesions and the changes in such lesions with time. In a study of various sorts of lesions produced by intradermal injections in rabbits, Gell and Hinde[1] attempted to isolate certain reaction-patterns which could be recognized in complex lesions. These were: 1) the vasculo-necrotic pattern, best recognizable in the passive Arthus reaction at 24 hours, which consists essentially of thrombosis of the small venules with platelets and white cells, leading to acute necrosis and massive invasion with polymorphonuclear leukocytes; 2) the "perivascular-island" pattern, best seen in a mild tuberculin reaction at 24 hours, but persisting in such sites for days or weeks, consisting of the "cuffing" of the small vessels with mononuclear cells, without any gross damage to the circulation or vessel; this picture is, however, identical with that seen in a mild Arthus reaction at three to four days, when the polymorphonuclear exudate has cleared; and 3) the "plasma-cell transformation" which occurs in Arthus reactions after five days and consists of the progressive transformation of the mononuclear cells to pyronin-positive cells and finally to typical plasma cells. The attempt was then made to follow the development of Arthus-type sensitivity histologically. Intradermal injections of full antigen (human gamma globulin) were made thrice weekly in rabbits and the lesions so produced examined macro- and microscopically. Microscopic examination of lesions at 24 hours showed that the first cell to appear as sensitivity developed was the mononuclear; by about the sixth injection, a pure perivascular island picture was produced indistinguishable from a "tuberculin" reaction. In subsequent lesions a central polymorphonuclear exudation, with vascular damage, became more and more evident, until finally this predominated, leaving only a peripheral zone of mononuclear reaction; it became, in fact, a typical active Arthus reaction. This suggested that the active Arthus reaction is a "vasculo-necrotic" type of reaction imposed

upon a "perivascular island" pattern of tuberculin type. Passive reactions produced by transfer to normal animals of serum or of blood cells, separately, showed that serum-sensitized animals tested intradermally with antigen showed an intense vasculonecrotic phase, while the "mononuclear" phase (best seen in active reactions at the third day) was inconsiderable; while cell-sensitized recipients showed a rather weak primary reaction, but a mononuclear reaction at three days which was disproportionately intense.

Experiments are being carried out in an attempt to identify the "sensitized" cell that enters the lesion, and to confirm the observation that such a cell matures to a cell resembling a plasma cell, by the injection of carbon particles before and after the establishment of various sorts of lesions. In general, it has been found that in an animal which has phagocytosed carbon and is then treated with antigen, a certain number of carbon-containing macrophages find their way into the lesion, presumably from spleen or bone marrow. If, however, carbon is injected into an animal as the lesion is appearing, the endothelial cells of the small venules become heavily loaded in their cytoplasm, and carbon-containing cells move out with the perivascular infiltrate in large numbers. The possibility is suggested that this is actually the result of proliferation of vascular-endothelial cells, rather than of the perivascular adventitial histiocytes, as is usually believed, and that vascular endothelium may be one specifically "sensitized" tissue. In the plasma-cell reaction in the late Arthus lesion, in carbon-injected animals, carbon may occasionally be found in mature plasma cells, suggesting that they have developed from macrophagic cells, but such experiments are still incomplete. As a working hypothesis, it is suggested that the cell that takes up antigen in small amounts may become "sensitized" and capable of taking part in a "delayed" reaction (though such a reactivity in Arthus-sensitized animals is usually suppressed by the vasculonecrotic phase): this "sensitized" cell when in the tissues and in the presence of retained antigen may then mature to an antibody-producing plasma cell.

*DISCUSSION*

MERRILL W. CHASE: We have heard tonight the net result of a broad area of research in which Isobel Hinde and our speaker have been engaged for so long, one in which the cellular reactions occurring within test sites on rabbits have been carefully quantitated by study of section after section; it represents a necessary pattern of work to which so few people are willing to devote the required amount of time and carefulness. From a fairly complex series of changes within the tissues, there have been sorted out certain patterns that are associated with Arthus-type reactions and with tuberculin-type reactions respectively; one pattern or the other can be discerned in rabbit lesions, and sometimes the participation of both simultaneously.

It seems to this discussor, whose work with drug allergy in guinea pigs has led him to differentiate more sharply between the two types of reactions (immediate and delayed) than Dr. Gell does, that the rabbit may not be an advantageous species for studying and differentiating between the varieties of tissue response. In this species, reactivity towards tuberculin after the sensitization is not especially high, old tuberculin in dilution of 1:5 to 1:100 being frequently required, whereas the Arthus-reactivity of the rabbit and its related concentration of circulating antibody are generally more pronounced than in other species. In consequence, a "ratio" of delayed to immediate hypersensitivities (expressive of the rabbit's capacity to exhibit the two effects) could be represented as a fraction having a small numerator and a large denominator. The reverse of this may describe the ratio of delayed to immediate hypersensitivities in the guinea pig, possibly even best with respect to sensitization with drugs: the delayed component is high, antibody production is low. Our points of view, therefore, may be conditioned by the evidence that has been available to us. From my own experience, which is based on experimental manipulation and not on histologic observation, I conclude that the delayed-type of drug allergy in the guinea pig is distinctive and differentiable from reactions of the "immediate" type, that is,

reactions referable to the classical types of circulating antibody.

For example, in certain specific test sites made on guinea pigs that have been appropriately sensitized, one can distinguish the co-participation of Arthus-type and delayed-type responses at the same site; further, by specific desensitization one can suppress or abolish the capacity to give Arthus-type reactions and leave the delayed-type of reactivity essentially unimpaired.

The studies which Dr. Gell presents are, therefore, paralleled in certain guinea pigs sensitized appropriately with selected chemical allergens, in that we can find either pattern occurring alone or both appearing together with some degree of linkage between the two.

One particular result that we have seen in the guinea pig merits specific reference, not because it contradicts but because it may be explained on the basis of Dr. Gell's observations with rabbits, with respect to his findings of vascular cuffing following a local test. When a guinea pig has been rendered hypersensitive to tuberculin, and a tuberculin test of moderate intensity has been made on the belly wall by means of the tuberculin preparation PPD, the animal exhibits the typical, delayed-type of reaction to tuberculin and the site then slowly reverts and approximates its normal state. (The intensity of the test has been arranged so as to avoid local sloughing, but to exhibit the characteristic shift from brawny edema at 24 hours to induration at 48 hours—characteristics that lead us to recognize this reaction as being a typical tuberculin reaction.) After recovery of the site, say three weeks later, 25 gamma of PPD are injected subcutaneously at a remote site. There ensues around these former test sites, commencing within some hours and reaching a maximum within 24 hours, a specific reaction that reveals the existence of a local immunologic alteration at these sites. This would fit very well with Dr. Gell's statement that monocytes persist for some time in a perivascular location at the site of tuberculin reactions conducted in the rabbit. Yet the new reaction is probably an anti-protein reaction, and not a delayed type of reaction. If my statement as to the sequence and interpretation of events is correct, the animal that has been caused to undergo a reaction of delayed type acquires at a test site not only what Inderbitzen[2, 3] has reported—an increased amount of histamine, detectable for several days and attributed to the ingress of cells—but also an immunologic activation, likewise probably owing to the localization of wandering cells, which appear to become engaged in antibody formation. When the tuberculin preparation PPD is supplied later in moderately high concentration at a remote point, it appears to reach the locally altered former test site and to induce there a reaction which is not a delayed type of reaction. My illustration should underline the complexity of the events as they occur *in vivo*. Decipherment of the component parts will not be an easy task.

I should like to point out still another situation which emphasizes some of the difficulties in interpreting, classifying, and understanding types of reactions. Dr. Janet McCarter, working at the University of Wisconsin, studied tuberculin tests in students. When it fell to her lot to use the first preparation of avian tuberculin that had been made with use of only minimal heating, followed by mild concentration on the ultrafilter, it was found that students who were negative in her initial test series responded transiently to later re-testing. The new type of preparation actually was antigenic. On the average, the maximum reaction occurred at 24 hours; some of the reactions persisted through the second day and would have been judged tuberculin-positive if observed only at that time. We are not required to accept the "positive" reactions, observed in re-testing, as being a delayed type of reaction, but we are obliged to recognize that the need to use minimal test strengths of tuberculin (in order to avoid focal and systemic reactions in highly sensitive individuals) and the bother of making step-wise tests on a patient often cause us to stop short of any critical appraisal of the types of skin reaction that we encounter in human skin-testing. When a reaction persists for a sufficient length of time for us to recognize it as positive we are likely to interpret it as a delayed type of reaction.

Dr. Gell has attempted to sort out the types of reactions on a histologic-biologic basis. Attempts of this sort must be pursued eventually with species other than the rabbit before all the necessary data come to hand. We are indebted to, and must congratulate Dr. Gell on the foresight that led to the work summarized here tonight.

A. R. MILLER, Seattle, Washington: If I understood correctly, Dr. Chase stated that monocytes become antibody-producing cells.

MERRILL W. CHASE: No, I did not intend to describe it so baldly; the cellular events in the perivascular area are probably complex indeed.

A. R. MILLER: I am wondering if it has to be a monocyte, or the cell which surrounds the antigen has to be the cell that will entertain the antigen and also would have to be the cell that produces the antibody. In other words, could it be a cell of a particular sort, such as the cell of the 8th nucleus of the pons that would take over the antigen-antibody reacting mechanism? I understood Dr. Chase to say that the cell became the antibody-producing agent, that the antigen itself, the PPD, was not the antigen in this reaction, so it seems to me the only way we could desensitize an individual would be in some way to extract the cells, make up an antigen of that, and desensitize to the cell itself.

MERRILL W. CHASE: The production of antibody is related to both splenic and non-splenic sources, and, as has been shown by Oakley, Batty and Warrack in England[4,5] and by others in this country, one can have local concentrations of antibody in different tissues; hence we can assume a local concentration of antibody in a node on one side of an animal and its practical absence contralaterally. The types of cells that are engaged in producing antibody are not precisely known, but it is generally regarded as a function of the pre-plasma cell. In my own opinion, the lymphocyte is at least part of an alternate parallel pathway. The lymphocyte does not necessarily play a complete role; possibly it provides some necessary material to other types of cells. This does not bear on anything that I have said, and I think it was Dr. Gell and not I who talked about plasma cells arising in such areas, in which there were concentrations of plasma cells and monocytes.

A. R. MILLER: I would like to ask Dr. Gell the same question.

PHILIP G. H. GELL: I am not sure that I understand it; will you please repeat your question?

A. R. MILLER: Let us take the 8th nucleus of the pons. Say we have a concentration of plasma cells around the vessels in this nucleus. Could it be possible that the nuclear cells themselves would take over the antigen, and also by retaining antigen itself produce antibody? In other words, your plasma cells and lymphocytes are left out of the picture.

PHILIP G. H. GELL: I do not think there is any conclusive evidence one way or the other at the moment; it is possible that a small amount of antibody or a small amount of gamma globulin may be capable of being produced in any cell in the body—it is certain only that the bulk of the antibody is produced in cells of the lymphoreticular system.

*REFERENCES*

1. Gell, P. H. G. and Hinde, I. T. Observations on the histology of the Arthus reaction and its relation to other known types of skin hypersensitivity, *Int. Arch. Allergy 5:* 23-46, 1954.
2. Inderbitzen, T. The effect of acute and delayed cutaneous allergic reactions on the amount of histamine in the skin, *Int. Arch. Allergy appl. Immunol. 7*:140-48, 1955.
3. Inderbitzen, T. The relationship of lymphocytes, delayed cutaneous allergic reactions and histamine, *Int. Arch. Allergy appl. Immunol. 8*:150-59, 1956.
4. Oakley, C. L., Batty, I. and Warrack, G. H. Local production of antibodies, *J. Path. Bact. 63*:33-44, 1951.
5. Batty, I. and Warrack, G. H. Local antibody production in mammary gland, spleen, uterus, vagina and appendix of the rabbit, *J. Path. Bact. 70*:355-63, 1955.

# *Homotransplantation of Human Cell Lines*

## *(Abstract)*

CHESTER M. SOUTHAM

Sloan-Kettering Institute for Cancer Research

The growth of established human cell lines after subcutaneous homotransplantation into healthy adult male volunteers and volunteers with advanced debilitating neoplastic disease has been studied[1]. The subcutaneous nodules which appeared at the inoculation sites were biopsied at various intervals from one week to eight weeks after implantation. Histologic findings were illustrated by lantern slides. Data illustrating the following observations and conclusions were presented.

Behavior of a given cell line on homotransplantation appears to be independent of its site of cultivation. For example, HEp #3 cells grown in tissue culture, egg chorioallantoic membrane, or cortisone-treated rats, mice, or hamsters have shown no apparent differences attributable to source.

Normal recipients have rejected implanted cells of all types. Regression is usually complete by three weeks, and some biopsies taken as early as seven days after implantation show no evidence of propagation or persistence of the implanted cells. Rejection in normal recipients was characterized, grossly and microscopically, by an acute localized inflammatory reaction at the implant site. Polymorphonuclear leukocytes were prominent in this reaction, but mononuclear cells including plasma cells were also present in large numbers, and eosinophiles were prominent in some specimens.

In the cancer patients rejection was delayed or did not occur at all during the period of observation. Acute inflammatory changes were minimal and transient or did not occur at all. Growth of the implanted cell (all cell types) was demonstrated in 20 of the 22 patients studied to date. Growth continued for two weeks or longer before there was regression in any of these patients, and in three individuals there was still active growth of the implanted cells at six weeks or later. When regression did occur it was characterized by degenerative changes of the implanted cells and a mononuclear cell infiltration with little or no polymorphonuclear response. If there was no spontaneous regression the study was terminated by complete excision of the implant or by expiration of the patients due to their own advanced cancer.

The several cell lines showed consistent differences in growth potential within both of the two categories of recipients (cancer patients and normals). Normal fibroblasts never gave any detectable evidence of propagation. Chang's conjunctival cells (epithelial cells of normal origin but showing hyperploidy and other cytologic abnormalities) never grew in normal recipients and grew slowly in the cancer patients. Most of the neoplastic cell lines (HEp #1, HEp #2, HeLa, J-111, and HS #1) formed a group which appeared to have about equal growth capacity. In normal recipients these cells were found in almost all biopsies taken on day 7, although they usually showed considerable cellular degeneration and were enmeshed in acute inflammatory tissue. In the biopsies taken on day 14 they were still demonstrable in somewhat less than half of the recipients. In the cancer patients these cells produced nodules of healthy-appearing cells in almost all recipients in the biopsies taken at two weeks or later after implantation, and in a few cases growth was progressive for three to eight weeks. The HEp #3 cell is in a group by itself. It was the most vigorous and aggressive of the cell types studied as judged by behavior in both groups of recipients. In

normal recipients all biopsies taken on day 7 contained HEp #3 cells although there was also a vigorous inflammatory reaction. About two-thirds of the biopsies taken on day 14 contained identifiable HEp #3 cells, although usually degenerative changes were prominent. In one normal recipient a nodule was still growing and contained cancer cells 21 days after inoculation. In the cancer patients growth of HEp #3 cell implants recurred three times after excisional biopsies and grew progressively in these three patients for six weeks or longer (that is until the patient died of the primary disease). In one of these individuals there was metastasis from the inoculation site on the forearm to the axillary nodes. In the other two cancer patients who received HEp #3 cell inoculation, one showed no recurrence after excisional biopsies on day 19 and the other died of his primary disease seven days after implantation.

In attempting to determine the cause of the difference in reaction between these two groups of recipients, an attempt is being made to investigate cellular and humoral defense mechanisms of all known types. Time does not permit a detailed discussion and these studies are still incomplete, but it may be stated that there appears to be no inability of the cancer patients to produce an acute inflammatory response to other types of stimuli and no defect in their ability to produce circulating antibodies against viral antigens. An interesting observation in the cancer group is the frequent occurrence of very low properdin levels. In the data accumulated to date there is a direct correlation between serum properdin levels and the observed regression of implants. However, there is neither evidence nor implication that this relationship is causal, and the possibility that there may be a relationship between properdin and transplant rejection has not yet been subjected to critical investigation.

The growth of a repeat implant of the same cell type has been studied in normal recipients. The repeated implants formed smaller nodules and regressed more rapidly as judged by gross and microscopic examination. This accelerated rejection of a second implant is presumably the result of an induced immunity. It has not yet been possible to study this phenomenon in patients with advanced cancer.

All of these studies concern only homotransplanted cells. There is no implication in our results or any of the statements concerning them that these observations have any carry-over to spontaneous cancer.

### *REFERENCE*

1. These studies have been possible only because of the cooperation and collaboration of many physicians, laboratory scientists and technicians, and volunteer recipients, at Memorial Cancer Center, Ohio State University, and Ohio State Penitentiary. The speaker acknowledges his indebtedness to these many co-workers. A preliminary report on some of these studies has been published: Southam, C. M., Moore, A. E. and Rhoads, C. P. Homotransplantation of human cell lines, *Science 125:* 158-60, 1957.

### *DISCUSSION*

HARRY S. N. GREENE, New Haven, Conn. I do not think that Dr. Southam need apologize for entering the domain of homotransplantation. The present extreme confusion in the field is due largely to the fact that people who are neither pathologists nor transplanters have worked so intensively in it.

As I understand it, the primary results of his experiments have been that cultured human cancer lines will not grow in normal individuals, but will grow in cancer patients. The subject of homotransplantation has been intensively studied, using legitimate experimental animals; the findings, which are similar to those cited, have been published and are well recognized. The success of homotransplantation of any tissue depends on two factors: first, the status of the tissue used for transfer, and second, the status of the host used, and this is particularly true in cancer. Cancer is, of course, not a sudden transformation of normal cells, but, on the contrary, represents the final stage in a developmental process. Throughout this process the morphology of the cells may remain completely unchanged, but tl tumor undergoes profound biologic changes, and these are expressed in transplantable reactions. If one divides the development of cancer into its two main phases, first, the phase in which the tumor is not metastasiz-

able, and second, the phase in which it is metastasizable, one gets two radically different sets of transplantation reactions. In the pre-metastasizable phase the tumor will grow when transplanted back elsewhere in the tumor-bearing individual, but it always fails to grow when transplanted to normal individuals, whether the normal individual is of the same species or of a different species. Let me repeat that: A tumor in a pre-metastasizable phase of development will grow when transplanted back elsewhere in a tumor-bearing individual. It always fails to grow when transplanted in a normal individual. In other words, at this stage of development the tumor is dependent for its continued existence and growth on factors peculiar to the tumor-bearing animal. These factors are not supplied by normal animals, and accordingly the tumor will not survive transplantation in normal animals. With continued development the neoplastic focus attains a new property, the ability to metastasize, and coincidently, it attains independence of the factors concerned in its genesis and development. Thus, a metastasizable tumor will grow not only on an autologous transfer, but it will also grow on transfer to normal, unrelated animals, even if the animals are of a different species. Let me emphasize this, using a particular tumor, a spontaneous breast cancer in one strain of my rabbits. It is a very constant tumor, the sequence of morphologic and biologic changes being the same in all cases. It begins as a cystic enlargement of the ducts and acini; over a period of time small foci of neoplasia occur in the dilated cysts and ducts. These always occur just as uniradicular papillomata, and then become multiradicular, and have the complicated structure seen in the present slide. A high power of this slide shows all of the cellular changes generally thought to be characteristic of cancer, that is, a considerable increase in the number of mitoses, loss of polarity, and marked irregularity in nuclear size and staining qualities. But for a considerable period of time the tumors remain confined within normal boundaries, and do not invade. If one obtains a biopsy at this stage of development and transplants fragments in the anterior chamber of the eye of normal rabbits, the transplants fail to take. This is a section of such a transplant three weeks after transplantation, — obviously dead. However, if fragments obtained from the same biopsy specimen are transferred to rabbits bearing spontaneous breast tumors the transplants survive and grow. In this stage of development the tumor is dependent for its existence on factors peculiar to the tumor-bearing host, and these factors are not supplied by the normal animal. With continued growth the primary tumor attains the ability to invade and metastasize, and if one transplants a fragment of tissue obtained at this stage one finds it will grow in normal animals. Not only does it grow in normal rabbits, but it grows in animals of any species, and the next slide shows the same tumor growing in the anterior chamber of the eye of a guinea pig. Thus when a tumor attains the metastasizable phase of development, it also attains independence of the factors it originally depended on, and will grow in their absence. This is not only true of tumors of rabbits, it is true of all cancers, whether in animals or man.

This slide shows the situation in a spontaneous carcinoma of a $C_3H$ mouse. A biopsy was performed on that tumor in March of one year. Fragments of the tumor grew on transfer to other $C_3H$ mice, but failed to grow in foreign mouse strains or in an alien species. The tumor was biopsied again a month later, and again fragments grew on autologous transfer, but not on homologous or heterologous transfer. When the animal was killed at the end of another month, small metastases were found in the lung. Transfer of the primary tumor was performed as in the previous experiments, but this time it grew on heterologous and homologous transfer, as well as in animals of the strain of origin. Thus the transplantation reactions of a tumor are entirely different in different developmental phases, and it is absolutely essential in any transplantation experiment involving cancer to characterize the inoculum. This point concerns human, as well as tumors of other species. The significance of heterologous transplantation and homologous transplantation is exactly the same, and in working with human tumors it is not necessary to use man as an experi-

mental animal. Let me illustrate this point. This slide shows the results of heterologous transfer of a series of 123 different human cancers obtained from the operating room. The tumors were all cancers morphologically. Most of them were carcinomas; there were a few sarcomas. Sixty-five grew in guinea pigs, and 58 failed to grow. In the group of tumors that were heterologously transplantable 64 of the patients are now dead; only one is alive, and this individual is moribund with metastases. Obviously these transfers were performed from patients in the terminal phase of the disease, and the subsequent rapidly fatal course of the patients indicates that in all probability their tumors had reached the metastasizable phase at the time of operation. On the other hand, in the group of patients with non-heterologously transplantable tumors only 12 have died and 46 are still alive and free of tumor. It would appear therefore that in this group the tumors had not attained metastasizability at the time of operation.

My point then is that the transplantability of human cancer depends on two factors: first, the status of the tumor tissue used, whether it is from a metastasizable cancer or a non-metastasizable tumor, and secondly, the status of the patient: whether normal or tumor-bearing. It would seem to me therefore imperative that in the work that has just been reported that the status of the tumor used be clarified.

I should also like to raise the point as to the necessity of using human beings in such an experiment as this. In some backwoods district of New England, remote from the sophisticated institutions of this City, we still believe in the dignity of man. That belief may be an illusion, and probably it is, but it is an illusion that surely should be preserved and fostered.

MERRILL W. CHASE: It seems to me that what we have heard tonight, if I interpreted it aright, is this: most of the materials with which Dr. Southam has been working have not been of the metastasizable sort, for they either were not effective as "takes" when supplied to normal individuals or they regressed rapidly. Speaking of these experiments as an immunologist, I wonder whether one cannot view them in this wise. Does Dr. Southam not have here a situation of immunologic tolerance in cancerous individuals to some class of antigens that is indigenous to cancerous cells, and of absence of tolerance (i.e., rejection) in normal individuals? Obviously we cannot picture simply the antigens of normal cells as meeting the needs of our hypothesis, because we have been told that normal fibroblasts are rejected by both classes of recipients. If we are going to pursue this line of reasoning, these antigens should be something special and common to an entire series of cancerous cells, and indeed many of the cell lines used were epidermoid in origin. Could these cancerous cells possess some special antigen or class of antigens, other than those present in normal cells? Could one picture some sort of immunologic tolerance in cancer patients, possibly connected with a high metabolic rate of the cancerous tissue, a liberation of certain antigen materials (nuclear in origin? present in the native state or as split products?) and a saturation of the chief antibody-producing sites?

If this were the case, possibly a kind of immunologic tolerance could be set up. A pertinent model would perhaps be the deviation of the usual event of isoallergic encephalomyelitis by special prior injections of brain tissue, now being studied by Dr. Philip Y. Paterson. Perhaps one could imagine, further, that the antigens involved in Dr. Southam's cell lines are only weak isoantigens, and that some engrafted kind of tolerance against this particular material might be established without too much difficulty. Reasons for the occurrence of such a special antigen or antigens in these cells would be beyond the scope of the hypothesis. One would have, rather, to seize upon Dr. Greene's experiences and his findings that cancerous cells undergo alteration with a maturing process and acquire special new properties of metastasizability. Indeed, it is well known that changes in ploidy occur in many of the cell lines that are maintained under artificial conditions.

It would be pertinent to inquire whether, when an animal has developed an increasing rate of rejection for a given cell line, there is concomitant change in the resistance of

this individual toward other cell lines. Let us rephrase the thought. Would the immunologic experience gained by contact with one human epidermoid cell line condition a person so as to determine his subsequent reaction to all epidermoid cell lines or only to the same epidermoid? Would his initial tolerance for another sort of cell, say a fibroblast, be entirely normal? Answers to these questions might provide some clue in looking for special antigens in cancerous cells. In other words, I would like to have Dr. Southam tell us more about the increase in rejections seen in the non-cancerous subjects.

I should also like to ask Dr. Greene whether he has some explanation for the change he finds to occur in cells that enter the metastasizable phase.

HARRY S. N. GREENE: That to me is the crux of the cancer problem; if the factors distinguishing metastasizable from non-metastasizable cells were known, I think we would be pretty close to the answer. I do not know what the factors are.

CHESTER M. SOUTHAM: Dr. Greene brought up three points that I would like to comment on. First, I would like to ask Dr. Greene if he classifies a specimen as metastasizable or pre-metastasizable on the basis of clinical and histologic characteristics at the time the specimen is obtained and then correlates this classification with its behavior on transplantation. Or, on the other hand, is a specimen classified only after observation of its growth in homologous and heterologous hosts, and then correlated with clinical behavior?

Second, Dr. Greene has suggested that the tumor-bearing animal is more receptive to transplants because the first tumor supplies something which is necessary for the growth of the transplanted tumor, but which is lacking in a normal recipient. In contrast, it has been my premise that the tumor-bearing animal lacks some defensive mechanism which the non-tumor-bearing animal has. While this may be quibbling, since either interpretation provides a useful working hypothesis, these different interpretations are of interest because they might lead to different experimental approaches to the investigation of these phenomena.

Before I discuss philosophical differences across the state border, I would like to consider Dr. Chase's question regarding the increased rejection rate of reimplants of the same or different cell type, and the possible implications of such studies regarding the antigenicity of the several cell types. I did not have time to present our data on repeat homotransplants but my statement concerning the more rapid rejection of second implants was based on two criteria. The first is smaller nodule size. Obviously, nodule size alone is a measure of reactive tissue as well as growth of the implanted cells, but for what it is worth, you see in this graph a plot of nodule size against the number of days after implantation. These studies were all in normal recipients.

In primary implants there was a definite nodule at day 7, maximum nodule diameter at day 14, little further change by day 21, but nearly complete disappearance by day 28. (The data illustrated concerned implants of HEp #2 or HEp #3 cells.) In a group of individuals who were inoculated simultaneously with the primary implant group, but who had received a previous implant of the same cell type some 3 to 4 months earlier, the nodules were at their maximum size at 7 days and were already regressing by day 14. In another group of individuals who had previously received an implant of a different cell type, there was little or no increase in nodule size after day 7, and regression was rapid. The number of individuals in each group is small I know, but that is what we have to work with. From these data I am suggesting the interpretation, but not insisting it is correct, that individuals who receive the same type of cell a second time reject it more rapidly than individuals receiving it for the first time, and that individuals who are having their first experience with a given cell type, but had previously received a different type of cell, may also show an acceleration of rejection, although perhaps not as great as in those who got the same type previously.

One other slide tackles this same question from a different angle. On this chart I

have attempted to quantitate roughly the amount of growth of the implanted cells and the severity of inflammatory reaction. (The data illustrated concerned HEp #3 cell implants only.) At 7 days after implantation all ten biopsies from primary implants contained considerable numbers of the transplanted HEp #3 cells; in the three biopsies from repeat implants of the same cell type, HEp #3 cells were present in only one, and when the HEp #3 cells had been implanted into individuals who had previously received implants of some other cell type, few biopsies (two out of 15) showed as good growth as in the primary implants, three contained no HEp #3 cells, and in the other ten the implanted cells were rare. Similarly in biopsies taken on day 14 most of the primary implants (eight out of 13) contained some HEp #3 cells, whereas in repeat implants of the same cell type the only nodule which had not completely regressed contained no HEp #3 cells, and likewise there were no HEp #3 cells in the two biopsies from recipients who got HEp #3 cells for the first time after previous implants of different cell types.

I am well aware that these data are not adequate to draw conclusions from, but they do suggest the possibility that some antigens are common to the several neoplastic cell types, as Dr. Chase said. I say antigen in quotes, because as yet we have not been able to demonstrate any circulating antibodies. Our only demonstration of an immune state has been this accelerated rejection.

Now to return to Dr. Greene. I think that in general it is fruitless to attempt a rebuttal in philosophical debate, but there are a couple of points here that must not go unchallenged. Dr. Greene—I am not quoting him exactly—is saying in essence that response to heterologous transplantation is not different from response to homologous transplantation and thus (if I am paraphrasing him correctly) we cannot expect to learn anything from the homologous transplantation (which requires human volunteers) which we could not learn from heterologous transplantation in laboratory animals. As a newcomer to the field of transplantation, I am not in a good position to refute this, but neither am I willing to accept it. It has been my assumption that in heterologous transplants the host is faced with tissues which are entirely foreign to it, whereas in homotransplantation some of the antigenic components of the transplanted tissue may be compatible with host antigens and hence might not elicit a rejection reaction. The possibility of continued growth of transplanted tissues has tremendous clinical interest both because of the possibility of using grafts to replace damaged vital structures, and because of the conceivable dangers of introducing into the human body transplanted cells which have neoplastic characteristics or potentialities. These studies of homotransplantation in man have been undertaken in the belief that experimental animal data in general, and results of human cell heterotransplantation studies in particular, could not be applied directly to man, and that the potential clinical importance of homotransplantation justified our request for human volunteers.

But be that as it may, the major point of philosophic difference is Dr. Greene's statement concerning the dignity of man. I would say that the best answer to this would be the attitude of those people who are involved in these experiments as recipients. What do the volunteers themselves think about their personal dignity? I have no idea how many of you have been inside of a prison, but after 16 visits I have come to the conclusion that the inside of a prison is very much like the outside of a prison, and that the individuals there have, at least in their own minds, just as much dignity and just as much individuality as we have here. They do not feel that it is an affront to their dignity, but rather they have demonstrated again and again that they feel it is a big thing which they are doing, or hope that they are doing, for man in participating in this type of work. Maybe some explanation will help. We initiated this project by putting an explanation of the work we wanted to do in the prison newspaper. We stated optimistically that we hoped to get 25 volunteers. Instead, 160 individuals volunteered within a week, and I do not know how many in the next month. There was no other publicity at that time. There was no

pressure or special inducements of any kind. The oniy reward for doing this was the bolstering of their personal sense of dignity. It is my personal belief that the greatest indignities that man can suffer are sickness and death. This work which we are doing as investigators, and in which these prisoners and patients are participating as volunteers, is predicated on the assumption that we are going to combat these indignities. Whether it will ever actually contribute to improvement in cancer treatment, or any other phase of therapeutics, we cannot even guess, but that is our aim. The idea that the participation of volunteers in such work is offensive to their personal dignity is completely foreign to my own thinking. In fact I feel that such altruistic service to mankind is a convincing demonstration of the dignity of man.

HARRY S. N. GREENE: I will not go further into the discussion of human dignity. Personally I do not feel that the discovery of the cause and cure of human cancer is worth the sacrifice of human dignity attendant on using man as an experimental animal.

The other question that Dr. Southam asked me was about how to determine whether the human tumors to be used were premetastasizable or metastasizable. That of course depends on the physical examination of the patient at the time of operation. If such is not conclusive, then the length of time the patient lives after removal of the tumor is a highly suggestive indication.

CHESTER M. SOUTHAM: You do not mean metastasizable, but metastasizing.

HARRY S. N. GREENE: The terms are probably synonymous.

JOHN G. KIDD: The question could be cleared up by a statement from Dr. Southam as to whether the cells of the growths with which he worked were cultured from growths that had already metastasized.

CHESTER M. SOUTHAM: The HEp #1, #2, and #3 patients are all dead and all had metastatic disease. HEp #1 came in 1953 from an inguinal node metastasis of epidermoid carcinoma primary in the cervix. Death occurred a few weeks after the biopsy was obtained and was due primarily to pelvic cancer causing uremia. HEp #2 was grown from the primary lesion, of an intrinsic larynx carcinoma. Total laryngectomy was performed and recurrence appeared successively in both sides of the neck during the next 15 months and was treated by radical neck dissection. Thereafter the patient had no clinical evidence of cancer. He died of bronchopneumonia at another hospital 27 months after the laryngectomy. At autopsy the only cancer was a metastasis in one adrenal. HEp #3 was grown from the primary lesion of epidermoid cancer of the buccal mucosa which had been diagnosed and treated by x-ray therapy 6 months prior to surgery. At the time the specimen was obtained the disease was recurrent in the cheek and metastatic in the nodes of the right neck at all levels. Postoperatively the disease progressed with extreme rapidity. There was massive pleural effusion positive for neoplastic cells, although chest x-rays were normal one day before operation. Signs of lumbar cord compression appeared and the patient died just two weeks after surgery. No autopsy was permitted.

These three epidermoid carcinomas thus appear to fit Dr. Greene's thesis that those lesions which grow on homo- or heterotransplantation are metastasizing cancers. In contrast, however, are the results with HS #1. This was cultivated from a well-demarcated sarcoma in the left calf muscle, apparently primary in that site. It was signed out as an unclassified sarcoma, possibly a liposarcoma. The only treatment was local excision in January 1953, and there has been no evidence of recurrent or metastatic disease according to the latest chart entry (July 1956). I did not discuss HS #1 very much in my presentation, but you may recall that in one of the cancer patients it grew for 57 days. In the normal recipients it behaved somewhat like HEp #3 although producing less intense inflammation. This tumor then, as well as Chang's conjunctival cells, seems to contradict Dr. Greene's thesis.

May I add, à propos of New England versus New York philosophy, and just for

the record, that I am from Salem, Massachusetts, where our good-intentioned but bigoted forebears hanged the witches.

HARRY S. N. GREENE: We just completed a long series of experiments on the influence of the first transplant on the second, and found there is not actually a resistance to the type of tissue we originally transplanted, but a resistance to transplantation itself.

MERRILL W. CHASE: Dr. Greene's last comment perhaps deals with transplantation into other species, and if so the situation would be different from that of isotransplantation as regards the classes of antigens that would come into prominence. As a basis for our thinking, we might take as a model transfusion accidents occurring during repeated transfusions in which proper account has been taken of the major blood group but not of the existence of minor blood group antigens (M, N, P, Rh, and many others). Reference is made to these erythrocyte antigens because they are well known to us in our daily experiences. Similar or additional antigens occur in sperm cells, in circulating white cells, and in tissue cells generally. Such antigens within fibroblasts are probably the basis for an immunologic reaction that results in rejection of implants of these cells by both cancerous and non-cancerous subjects alike. What then of the inertness of the cancer patient towards human epidermoid cancer cells? There must be an explanation for the presence of a positive anergy that tolerates epidermoid cells but not the antigens of fibroblasts. Such tolerance ought to rest, one would suppose, on some antigen that is dominant in and peculiar to epidermoid cancer cells, being dominant even over antigenic components of normal tissue cells. It would require, also, some degree of related antigenicity between all epidermoid cell lines. The new evidence provided by Dr. Southam suggests that cross-reactions are, indeed, exhibited in view of the suppression that obtains when other epidermoid cell lines are used later on.

Following desensitization the once anaphylactically-sensitized guinea pig can be maintained in the desensitized state providing that we keep supplying enough antigen, for the animal's tissues cannot dispose of the excess antigen. If cancer cells had a property of liberating enough of a special antigen, perhaps a positive "anergy" could be established and maintained in somewhat similar fashion.